

PROTEASE INHIBITORS IN MAMMALIAN BLOOD SERUM
AND MAMMARY SECRETIONS
WITH SPECIAL REFERENCE TO THEIR FUNCTION
IN THE GROWING PIG

BJÖRN R. WESTRÖM



LUND 1981



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SECRETIONS WITH SPECIAL REFERENCE TO THEIR FUNCTION IN THE
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av

BJÖRN R. WESTRÖM

FK., Mln

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Title and subtitle Protease inhibitors in mammalian blood serum and mammary secretions with special reference to their function in the growing pig.							
Abstract Protease inhibitors were identified and characterized in porcine blood serum, amniotic fluid and mammary secretions with emphasis on the development and physiological function during ontogeny of the pig. At least seven protease inhibitors were identified in adult porcine serum based on differences in mol.wt., electrophoretic mobility, protease inhibition specificity and immuno-cross-reactivity with human protease inhibitors. Apparent similarities exist between the porcine and human protease inhibitors and those of other mammalian species. However, some differences are also evident i.e., the existence of two α-macroglobulins and an apparently specific trypsin inhibitor, α_2-antitrypsin in the pig. High levels of α_1-protease inhibitor and α_2-antitrypsin in fetal serum and amniotic fluid and their decrease in serum immediately after beginning colostrum ingestion after birth, suggest that the inhibitors have important functions. Since, intestinal absorption of undegraded proteins takes place in the piglet during this period, protection from intestinal proteases by these inhibitors seems probable. The high protease inhibiting capacity in porcine colostrum is mainly due to the specific colostrum protease inhibitor, SCTI, but 10-20% of the inhibition comes from serum-type inhibitors. The inhibition rapidly decreases in milk during the first days of lactation and after 5 days only 1% of the original inhibition remains and no SCTI was detected. The protease inhibitors in milk, primarily those of serum-type, constitute a protection of the mammary gland from proteolytic attack, e.g., during colostrum formation and mastitis. By feeding newborn piglets colostrum in which the protease inhibition was eliminated by the addition of trypsin, it became evident that the colostrum protease inhibitors prevent the degradation of proteins in the gut, resulting in enhancement of the intestinal transmission of undegraded colostrum proteins, such as albumin, IgG and β-lactoglobulin to the blood circulation.							
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From the Department of Zoophysiology,
University of Lund,
Lund, Sweden

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This Thesis is based on the following papers which will be referred to by their Roman numerals, I-V, and on some additional unpublished observations.

- I. Weström B.R. (1979) Protease inhibitors in porcine serum and their immunological relationships to human protease inhibitors.
Hoppe-Seyler's Z. Physiol. Chem. 360, 1861-1867.
- II. Weström B.R. (1979) Identification and characterization of trypsin, chymotrypsin and elastase inhibitors in porcine serum. Hoppe-Seyler's Z. Physiol. Chem. 360, 1869-1878.
- III. Carlsson L.C.T., Weström B.R. & Karlsson B.W. (1980) Intestinal absorption of proteins by the neonatal piglet fed on sow's colostrum with either natural or experimentally eliminated trypsin-inhibiting activity. Biol. Neonate 38, 309-320.
- IV. Weström B.R., Karlsson B.W. & Svendsen J. (1981) Levels of serum protease inhibitors during fetal and postnatal development of the pig. Biol. Neonate 00, 000-000.
- V. Weström B.R., Svendsen J. & Karlsson B.W. Protease inhibitor levels in porcine mammary secretions. Submitted for publ.



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1. INTRODUCTION AND PURPOSE OF THIS STUDY

Protease inhibitors of animal origin are proteins capable of blocking the catalytic site of proteolytic enzymes, thus inhibiting the activity and regulating the action of these enzymes in the organism. Since proteases are participants in most important biological processes, such as protein metabolism, blood coagulation, fibrinolysis, inflammation, immunodefence, fertilization and protein breakdown in the gastrointestinal tract, protease inhibitors can serve as regulators in these systems, limiting the action of the proteases to the biological requirements.

It was the area of protein breakdown in the gastrointestinal tract that aroused our interest in the action of protease inhibitors in the growing pig. The remarkable feature of the pigs and other ungulates, whereby they postnatally obtain passive immunity from the early milk, colostrum, has long been known (reviewed by Brambell, 1970), but the mechanism by which the antibodies could escape proteolytic breakdown in the gastrointestinal tract has remained obscure. It was not until Laskowski & Laskowski (1951) detected a trypsin inhibitor in cow colostrum, BCTI, and subsequently in sow colostrum, SCTI (Laskowski et al., 1957), that the first indications were obtained. Several attempts have been made to prove the importance of SCTI in the absorption of undegraded proteins, but they have not been fully conclusive (reviewed by Jensen, 1978). Therefore, one aim of this investigation was to study the effect of SCTI on the absorption of proteins from the intestinal tract of the newborn piglet. This was done by adding trypsin to sow colostrum, thus abolishing its inhibiting capacity (III). Some evidence has also been obtained that SCTI is not the only protease inhibitor present in porcine colostrum and milk (Weström et al., 1979). Inhibitors similar to those found in the serum also appear to be present in mammary secretions, and a study was undertaken to characterize the development of SCTI and the serum-type inhibitors in porcine mammary secretions throughout the lactation period (V).

Only limited investigations into the existence and function of protease inhibitors in the blood serum of mammals other than man have been performed. However, trypsin, chymotrypsin and elastase inhibiting activities have been found in porcine serum (Carlsson & Karlsson, 1972; Nakamura et al., 1972; Baumstark, 1973). Therefore, it was decided to characterize

the protease inhibitors in porcine blood serum, in order to obtain a better understanding of their composition and physiological function, especially during the perinatal development of the pig. The porcine inhibitors were characterized both by studying the immuno-cross-reactivity with human protease inhibitors, which are well characterized (I), and by investigating the protease, e.g., trypsin, chymotrypsin and elastase inhibiting properties of porcine serum (II).

A remarkably high trypsin inhibiting capacity in the serum during porcine fetal development, both when compared to the adult pig and when compared to human fetal levels, had been previously observed (Carlsson & Karlsson, 1972). This high inhibiting capacity rapidly decreases after birth, when the piglet begins to ingest colostrum. Thus, the development of the individual serum protease inhibitors during the ontogeny of the pig was also studied, in order to obtain some insight into their role during porcine development (IV).

In addition, a survey of the protease inhibitors in blood serum and mammary secretions of the mammals was made and is presented in this Thesis, to provide a basis for comparison with the results obtained for the pig. The comparative aspects are especially examined in an attempt to elucidate the relationship between the mode of protein transmission between the mother and her offspring, e.g., via postnatal intestinal transmission, and the pattern and development of protease inhibitors in different groups of mammals.

2. CHARACTERIZATION OF THE PROTEASE INHIBITORS IN MAMMALS

2.1 Blood serum inhibitors (see also Table 1)

Man is the species most thoroughly studied with regards to the protease inhibitors found in the blood, and therefore, reference to these human inhibitors is often made when studying the inhibitors in other species. At least seven protease inhibitors are now recognized, comprising approximately 10% of the proteins in human plasma. A number of extensive reviews on the human plasma protease inhibitors have been published (Heimbürger, 1975; Laurell & Jeppsson, 1975; Harpel & Rosenberg, 1976; Starkey & Barrett, 1977; Collen & Wiman, 1978). Therefore, only a brief summary will be given here, without noting the individual references.

The high molecular weight protease inhibitor α_2 -macroglobulin, mol. wt. 725.000, is present in human plasma in a concentration of about 2 mg/ml, or 3.5 μ M. α_2 -Macroglobulin has a carbohydrate content of 8%, and a mean isoelectric point of pH 5.4. Due to genetic polymorphism and differences in the carbohydrate moiety, α_2 -macroglobulin shows some microheterogeneity upon electrophoresis. α_2 -Macroglobulin dissociates into two half-molecules (pair of chains) upon denaturation, and into four single chains, each having a molecular weight of about 190.000, upon reduction of the disulfide bridges. Each half molecule appears to be able to bind a protease molecule, thus the binding ratio for most proteases is 1:2. α_2 -Macroglobulin inhibits proteases of all the four functional classes, and thus shows a wide spectrum of inhibition. The liver may be the site of synthesis of α_2 -macroglobulin, but there is some indication that it may also be synthesized by monocytes and fibroblasts (Hovi et al., 1977; Cassiman et al., 1980).

Inter- α -trypsin inhibitor has a molecular weight of 160.000, and migrates in the inter- α -region upon electrophoresis, as indicated by its name. This inhibitor shows inhibition activity against trypsin, but only weakly inhibits chymotrypsin. Since this inhibitor is only present in low concentrations of about 0.4 mg/ml in plasma, and only has a low relative affinity for the above-named proteases, inter- α -trypsin inhibitor must be of limited importance as an inhibitor of these proteases in human plasma. Inter- α -trypsin inhibitor appears to be a precursor of certain low-molecular weight protease inhibitors present in plasma, urine and bronchial

mucus. The inhibitor contains two domains which are homologous with the Kunitz-type inhibitors, i.e., bovine basic pancreatic inhibitor and cow colostrum inhibitor (Hochstrasser et al., 1976).

C 1-Inactivator is an α_2 -globulin which has a molecular weight of 105,000. This inhibitor is especially rich in carbohydrates, having a content of 35%. Low levels of C 1-inactivator, about 0.25 mg/ml, can be found in human plasma. It can inhibit the activation of proteases in the proteolytic systems in the blood, e.g. coagulation, fibrinolysis, complement, kallikrein-kinin.

α_1 -Protease inhibitor (α_1 -antitrypsin) is a major human protease inhibitor which occurs at a concentration of 2 mg/ml or 50 μ M in plasma. It has a single polypeptide chain containing 12% carbohydrate and has a molecular weight of about 50,000-55,000. α_1 -protease inhibitor shows a pronounced microheterogeneity, and its average isoelectric pH is 4.8. In addition, α_1 -protease inhibitor shows extensive genetic polymorphism, and individuals having certain of these genetic variants, i.e., are homozygous for Z variant, have a low plasma inhibiting capacity and are predisposed to lung emphysema and liver cirrhosis. Trypsin, chymotrypsin, elastase, collagenase and plasmin are all inhibited by α_1 -protease inhibitor in a molar ratio of 1:1. α_1 -Protease inhibitor only reacts with active proteases by blocking their active site, followed by a cleavage of the inhibitor (Johnsson & Travis, 1976; Oda et al., 1977). The liver is probably the major source of α_1 -protease inhibitor, but the pancreas has also been implicated in its synthesis.

Antithrombin III is an α_2 -glycoprotein containing about 13% carbohydrate and occurring in rather low amounts in human serum, about 0.2-0.3 mg/ml or 4 μ M. Antithrombin III has a molecular weight of 65,000, and is thus slightly larger than α_1 -protease inhibitor. The inactivation of proteases participating in blood coagulation, such as thrombin and factor X_{α} , and of other proteases such as plasmin and trypsin, has been observed for antithrombin. The inactivation of thrombin is enhanced by the interaction of heparin with antithrombin III, probably by inducing a conformational change in the inhibitor, making the reactive site of antithrombin more accessible to thrombin. A deficiency of this protease inhibitor in humans leads to recurrent thromboses.

The third inhibitor eluted in the albumin peak upon the gel filtration of human plasma, α_1 -antichymotrypsin, has a molecular weight of 69.000, a rather high carbohydrate content of 25%, and an electrophoretic mobility in the slow α_1 -region. Rather low amounts of this inhibitor, about 0.5 mg/ml, are present in plasma, but this level rapidly increases upon the presence of inflammation, and it acts as an acute phase reactant. α_1 -Antichymotrypsin shows appreciable inhibiting activity only against chymotrypsin.

Recently, a fourth inhibitor, α_2 -antiplasmin has also been isolated from the albumin peak upon gel filtration of human plasma. This inhibitor has a molecular weight of about 65.000-70.000, and is present in plasma in low levels, about 0.07 mg/ml, or 1 μ M. α_2 -Antiplasmin is a single chain glycoprotein having a carbohydrate content of 12-14% and α_2 -globulin electrophoretic mobility. Besides inhibiting plasmin, this protease inhibitor also shows some trypsin inhibiting activity.

2.1.1 In the pig

Upon starting this investigation on the porcine protease inhibitors, gel filtration in combination with electrophoresis was used to survey and partially characterize the inhibitors in the various fluids studied. Agarose gel electrophoresis at pH 8.6 in combination with specific staining of the gels to detect the separated protease inhibitors proved to be of special advantage (II). Location of protease inhibiting activity was performed using both high- and low-molecular weight substrates, which gave complementary information. The high-molecular weight substrates, casein and elastin, were incorporated in the agarose gels, and after electrophoresis, trypsin, chymotrypsin and elastase, respectively, were applied to the gels. Inhibition was revealed as zones of undigested substrate. Excellent separation of the protease inhibitors in agarose gels was also achieved with the modified method of Uriel & Berges (1968), using the chromogenic substrate acetyl-DL-phenyl-alanine- β -naphthyl ester for visualization of trypsin and chymotrypsin inhibitors (II).

Another approach to identifying and characterizing the porcine protease inhibitors was to study their immunological relationship with the human serum protease inhibitors, which are well-characterized and to which

specific antisera were available (I). The cross-precipitates obtained between antisera to the human inhibitors and the corresponding porcine protease inhibitors were used to immunize rabbits; thus, it was possible to obtain specific antisera to the porcine inhibitors without prior isolation of these inhibitors. Using antisera specific to the porcine inhibitors, absorption experiments were carried out in order to correlate the inhibitors characterized using biochemical methods with those identified using immunological methods (II).

An α_2 -macroglobulin inhibitor was the first protease inhibitor to be identified in porcine serum (James, 1965; Jacquot-Armand & Guinand, 1967). Although the inhibitor showed two separate precipitates in immunoelectrophoresis (Jacquot-Armand & Guinand, 1967), it was not until recently that it was established that porcine serum contains two α_2 -macroglobulins having very similar properties (I, II, Martinsson & Carlström, 1977). They are named according to their slightly different mobilities in the α_2 -region of agarose gel electrophoresis, α_2 -macroglobulin f (fast) and α_2 -macroglobulin s (slow) (I). The designation pregnancy zone protein has also been proposed (Martinsson & Carlström, 1977) for the faster moving α_2 -macroglobulin because of its cross-reactivity with human pregnancy zone protein. However, this protein is normally present in high concentration in the pig, not just during pregnancy, therefore this name seems to be inappropriate. Similar to the macroglobulins found in other species, the porcine α -macroglobulins are large proteins with a molecular weight of 740.000-960.000 (Jacquot-Armand & Guinand, 1967; Tsuru et al., 1975), and they have a broad protease specificity (II, Tsuru et al., 1975; Jacquot-Armand & Guinand, 1976). α_2 -Macroglobulin f dominates in adult porcine serum, comprising about 75% of the α -macroglobulin fraction (Weström unpubl.). The porcine α_2 -macroglobulins cross-react with human, dog, and rat α_2 -macroglobulins, including human pregnancy zone protein (Weström et al., in prep.).

Two molecular forms of inter- α -trypsin inhibitor are present in porcine serum (I, II). The major fraction has an apparent molecular weight of 160.000 and inter- α -mobility in agarose gel electrophoresis, whereas the minor fraction shows a lower molecular weight of about 130.000, and has albumin mobility. These two forms of inter- α -trypsin inhibitor show partial immunological identity, and the minor form probably represents a native fragment of the larger form in the serum. Inter- α -trypsin inhibitor

shows inhibiting activity against both trypsin and chymotrypsin. Porcine inter- α_1 -trypsin inhibitor is immunologically related to human inter- α -trypsin inhibitor.

α_1 -Protease inhibitor has an apparent molecular weight of 51.000, and is the main inhibitor found in the albumin- α_1 -region of porcine serum (I, II). Since α_1 -protease inhibitor shows inhibiting activity against trypsin, chymotrypsin and elastase (I, II, Baumstark, 1973; Juneja & Gahne, 1981), this name is preferred to the other designation, α_1 -antitrypsin, which has been used by Martinsson & Carlström (1977) and Dziegielewska et al. (1980). There are two genetic forms of α_1 -protease inhibitor which have similar inhibition spectra (I, II, Juneja & Gahne, 1981). Porcine α_1 -protease inhibitor is immunologically related to human α_1 -protease inhibitor (α_1 -antitrypsin).

Another protease inhibitor in porcine serum that has α_2 -mobility on agarose gel electrophoresis, but has a lower molecular weight than that of α_2 -macroglobulin, about 60.000, is the inhibitor designated α_2 -anti-trypsin because of its apparent specificity for trypsin. α_2 -Antitrypsin appears in several electrophoretic fractions in the α_2 -region, and thus may correspond to the porcine serum protease inhibitor that shows genetic variation, in addition to α_1 -protease inhibitor (Juneja & Gahne, 1981). α_2 -Antitrypsin has a low relative affinity for trypsin when trypsin is added to adult serum, and probably it is of minor physiological significance as a trypsin inhibitor in adult porcine serum (Ohlsson et al., 1981).

A serum inhibitor migrating in the β -region on agarose gel electrophoresis which appears to be specific for elastase has also been identified (II, Baumstark, 1973). This inhibitor, called β -elastase inhibitor (II), has a molecular weight slightly larger than α_1 -protease inhibitor, in the range of 50-60.000 (II) to 80.000 (Baumstark, 1973). Porcine proteins showing immunological relationship to the human serum protease inhibitors antithrombin III and α_2 -antiplasmin have also been found in blood serum (I, II, Koide, 1979). Porcine antithrombin III has also been isolated, and consists of a single chain glycoprotein containing 16% carbohydrate and having a molecular weight of 58.000 (Koide, 1979).

2.1.2 In the horse

An α_2 -macroglobulin inhibitor has been characterized in equine serum (Matthews, 1979; Kueppers, 1980). Using various methods of electrophoresis, Matthews (1979) could separate this α_2 -macroglobulin into two or three fractions. Whether these fractions represent different α_2 -macroglobulin inhibitors or one and the same heterogeneous inhibitor is unclear.

Other inhibitors, called Pr, Pi 1, α_1 -antitrypsin and α_1 -protease inhibitor by different investigators, have been characterized in horse serum. These inhibitors all show properties similar to those of human α_1 -protease inhibitor (Ek, 1977; Juneja et al., 1979; Matthews, 1979; Kueppers, 1980; Pellegrini & von Fellenberg, 1980). Probably these inhibitors constitute one and the same inhibitor, equine α_1 -protease inhibitor, which shows genetic polymorphism in the albumin- α_1 -region upon electrophoresis. The α_1 -protease inhibitor has recently been purified, but showed some heterogeneity with regards both to molecular weight and electrophoretic mobility. However, this could be due to the isolation procedure used (Pellegrini & von Fellenberg, 1980).

Antithrombin III having a molecular weight of 52.500 and α_2 -mobility on electrophoresis, has been isolated from horse plasma (Kurachi et al., 1976). Equine antithrombin III showed both immunological and structural relationship to human and bovine antithrombin III.

Another equine protease inhibitor showing trypsin, but probably not chymotrypsin inhibition, has been identified and named, variously, Pi 2 (Juneja et al., 1979), Xc (Matthews, 1979), α_2 -antitrypsin (Kueppers, 1980), and α_2 -protease inhibitor (Pellegrini & von Fellenberg, 1980). This inhibitor probably is the same one in all these studies. It appears to have α_2 -mobility and a somewhat higher molecular weight than equine α_1 -protease inhibitor. However, only partial inhibition of trypsin was observed with this inhibitor when low-molecular weight substrates were tested (Matthews, 1979). Matthews (1979) also found indications of the presence of an equine inter- α -trypsin inhibitor upon examination of the various fractions obtained from the gel filtration of horse serum.

2.1.3 In cattle

An α_2 -macroglobulin having a molecular weight of 800.000 has been isolated from bovine plasma. It showed inhibition against a broad spectrum of proteases (Nagasawa et al., 1970a, b; Sugihara et al., 1971).

A bovine α_1 -antitrypsin has been purified, having a molecular weight of 45.000 and an isoelectric point of pH 4.0. This α_1 -antitrypsin also has a broad inhibition spectra, and thus appears to be similar to human α_1 -protease inhibitor (Tan & Gans, 1972). The acid-labile protease inhibitor having α_1 -mobility in electrophoresis and a broad inhibition spectra, that has been isolated by Wu & Laskowski (1960) and Habermann (1968), probably corresponds to α_1 -antitrypsin, although a somewhat higher molecular weight has been reported.

Bovine antithrombin III has α_2 -mobility and a molecular weight of about 56.000 (Kurachi et al., 1976; Nordenman et al., 1977). Antithrombin III was found to be heterogeneous, having isoelectric points that ranged between pH 4.5 and 5.0. Nordenman et al. (1977) found no immunological relationship between bovine and human antithrombin III, whereas Kurachi et al. (1976) did find evidence of such a relationship between human and bovine antithrombin III. Studies on bovine antithrombin III suggest that the mechanism of interaction between antithrombin III and the protease involves proteolytic cleavage of the active site of the inhibitor, similar to the interaction between α_1 -protease inhibitor and proteases (Wallgren et al., 1981).

Using two-dimensional gel electrophoresis, Juneja & Gahne (1980b) found three protease inhibitors in bovine plasma, Pi 1-3, in addition to antithrombin III. The relationship of these inhibitors to the above-mentioned α_1 -antitrypsin and the protease inhibitor isolated by Gray et al. (1960), is unclear. The existence of an inhibitor other than α_2 -macroglobulin and α_1 -antitrypsin that shows inhibiting activity against chymotrypsin has been reported by Habermann (1968). The observed genetic polymorphism of Pi-2 is similar to that of α_1 -antitrypsin, but Pi-2 does not show any appreciable inhibition of chymotrypsin, which is common for the α_1 -antitrypsins found in other species (Juneja & Gahne, 1980b). There is a possibility that there is also a bovine inter- α -trypsin inhibitor, since Hochstrasser

et al. (1976) could isolate an acid-stable trypsin inhibitor of the Kunitz-type from trypsin treated bovine serum (see also 2.1).

2.1.4 In the dog

Two distinct canine macroglobulin inhibitors have been isolated designated, α_1 -macroglobulin and α_2 -macroglobulin, according to the differences in their migration properties in the α_2 -region on agarose gel electrophoresis (Ohlsson 1971a). Both inhibitors have similar physiochemical properties and similar spectra of protease inhibition, both being able to inhibit trypsin, chymotrypsin, elastase and neutral leucocyte proteases (Ohlsson, 1971a, b, c). Although α_1 - and α_2 -macroglobulin are immunologically distinct, there is some relation between them because both proteins cross-react with human α_2 -macroglobulin, when using rabbit antisera. These dog α -macroglobulins also show some relationship to porcine and rat α -macroglobulins (Weström et al., in prep.). The electrophoretically fast macroglobulins, α_1 -macroglobulin in canine serum, and α_2 -macroglobulin f in porcine serum, appear to be antigenically closely related, while the same holds true for the respective slower macroglobulins (Weström et al. in prep.).

A canine α_1 -protease inhibitor (α_1 -antitrypsin) has also been characterized and isolated (Ohlsson, 1971d; Abrams et al., 1978; Schnizlein et al., 1980). This inhibitor appears similar to human α_1 -protease inhibitor with regards to molecular weight (57.000-58.000), electrophoretic mobility (α_1 -region), isoelectric points (pH 4.4-4.5), and broad inhibition of proteases. However, no cross-reactivity between human and canine α_1 -protease inhibitor was found (Abrams et al., 1978).

Canine antithrombin III has also been purified, having a molecular weight of 77.000 and acting both as a progressive thrombin inhibitor and heparin cofactor (Damus & Wallace, 1974). Juneja et al. (1981) demonstrated the presence of three protease inhibitors, Pi-1, 2 and 3, in canine serum, with the aid of two-dimensional electrophoresis. Pi-1 is apparently identical to the above-described α_1 -protease inhibitor, as indicated by inhibition spectra and genetic polymorphism, since Pi-1 shows three forms upon electrophoresis. Pi-2, which only showed appreciable trypsin inhibition

and Pi-3 are hitherto unidentified protease inhibitors found in canine serum.

2.1.5 In the rat

Two α -macroglobulin inhibitors have been identified and isolated from rat blood serum (Boffa, 1964; Ganrot, 1973; Gauthier et al., 1974; Gauthier & Mouray, 1976; Gordon, 1976; Weström & Carlsson, 1976; Nieuwenhuizen et al. 1979), but only one of these macroglobulins, α_1 -macroglobulin, is present under normal physiological conditions. The other inhibitor, as indicated by its name, α_2 -acute phase macroglobulin, is present only in large amounts during such conditions as injury, inflammation, pregnancy, and during fetal and neonatal life (Heim, 1962; Ganrot, 1973). These inhibitors have very similar physiochemical and protease inhibition properties. However, as indicated by their respective names, they differ in electrophoretic mobility and in isoelectric points. The rat α -macroglobulins are immunologically related to the human, canine and porcine α -macroglobulins (Gauthier & Mouray, 1975; Weström et al. in prep.).

An inhibitor having an intermediate molecular weight and α_1 -mobility has been found in rat serum (Huttunen & Korhonen, 1973; Weström & Carlsson, 1976; Gauthier & Ohlsson, 1978). Recently, this inhibitor was purified (Gauthier & Ohlsson, 1978; Esnard & Gauthier, 1980) and named α_1 -inhibitor III because it was the third inhibitor, besides α_1 -macroglobulin and α_1 -protease inhibitor, found that migrated in the α_1 -region of rat serum. It has a molecular weight of 215.000 and shows inhibition of both anionic and cationic trypsin, anionic and cationic chymotrypsin, and elastase. However, the inhibition is not complete, and the enzymes are apparently still partially active against both low- and high-molecular weight substrates, after binding to α_1 -inhibitor III. This inhibitor is regarded to be homologous to human inter- α -trypsin inhibitor because of their similar properties and immunological cross-reactivity (Gauthier & Ohlsson, 1978). Another trypsin inhibitor having similar molecular weight but having α_2 -mobility in agarose gel electrophoresis, has also been demonstrated in rat serum (Weström & Carlsson, 1976).

The pattern of the rat protease inhibitors having a molecular weight similar to that of albumin, i.e., those eluted in the third Sephadex G-200 peak, seems to be rather complex (Rosenberg et al., 1976; Weström & Carlsson, 1976; Hojima et al., 1977). Trypsin inhibition has been located in the α_1 -, α_2 - and β -regions upon agarose gel electrophoresis of rat serum (Weström & Carlsson, 1976). α_1 -Protease inhibitor (α_1 -antitrypsin), a homologue to human α_1 -protease inhibitor, appears to correspond to the fastest moving of these three inhibitors (Gauthier et al., 1978; Takahara et al., 1980; Ikehara et al., 1981). α_1 -Protease inhibitor has a molecular weight of approximately 50.000, and is microheterogeneous, having isoelectric points of pH 4.5-4.8. Trypsin, chymotrypsin and elastase can be inhibited by this protein.

Rat antithrombin III has also been isolated from rat plasma; it has a molecular weight of 63-64.000, and an isoelectric point of pH 4.85 (Koide, 1979; Kumada & Abiko, 1980). Both α_1 -antitrypsin and antithrombin III are probably synthesized by rat liver (Rowley and Miller, 1975; Koj et al., 1978). Recently, α_2 -antiplasmin, a primary plasmin inhibitor of rat plasma, was identified in the ascending portion of the third peak obtained from gel filtration of rat plasma; it has a molecular weight of approximately 90.000 (Högstorp et al., 1981). Rat α_2 -antiplasmin shows essentially the same properties as human α_2 -antiplasmin, but the two proteins do not immunologically cross-react. It is not clear if rat antithrombin III or rat α_2 -antiplasmin corresponds to any of the inhibitors having α_2 - or β -mobility which have been identified by Weström & Carlsson (1976). The inhibitor characterized by Hojima et al. (1977) appeared to correspond to the α_2 -inhibitor. Further investigation of the inhibitors found in the third peak eluted from Sephadex G-200 gel filtration are needed in order to obtain a more complete picture of these inhibitors.

2.1.6 In the rabbit

Two α -macroglobulin protease inhibitors have been isolated from rabbit blood serum (Picard et al., 1964; Got et al., 1965). These show similarities to the α -macroglobulins found in other species, and to each other, but they have different electrophoretic mobilities, and are named,

accordingly, α_1 -macroglobulin and α_2 -macroglobulin. During conditions where there is inflammation, α_1 -macroglobulin acts as an acute-phase reactant, and its concentration increases (Lebreton de Vonne et al., 1970). Rabbit α_1 -macroglobulin cross-reacts with human α_2 -macroglobulin, whereas rabbit α_2 -macroglobulin does not. Both rabbit macroglobulins also cross-react with a protein found in normal rat serum, presumably α_1 -macroglobulin (Berne et al., 1971). α_1 -Macroglobulin is synthesized by the isolated perfused rabbit liver (Mouray et al., 1965).

Rabbit α_1 -antitrypsin has been isolated; it has a molecular weight of 58.000 and multiple isoelectric points of pH 4.4-4.9 (Koj et al., 1978). Two major α_1 -antitrypsin components, designated F and S, which show immunological identity, could be separated by polyacrylamide gel electrophoresis. It has been suggested that rabbit α_1 -antitrypsin derives from two sets of autosomal genes, since the F and S forms behave as metabolically independent proteins (Regoezsi et al., 1980). In addition, rabbit antithrombin III has been isolated, showing close similarity to the antithrombins found in other species, but no immunological relationship to human, porcine or rat antithrombin has been detected (Koide, 1979).

2.1.7 In other mammals

An α_2 -macroglobulin protease inhibitor has been isolated both from the mouse (Greene et al., 1971), and from the hamster (Stein-Streilein & Hart, 1981). In addition, two antigenically distinct α_1 -antitrypsins (f and s) have been isolated from mouse serum (Myerowitz et al., 1972; Cauldie et al., 1980). These mouse α_1 -antitrypsins had slightly different electrophoretic mobilities and isoelectric points.

A guinea pig serum C 1-inactivator has been isolated; it has a molecular weight of 170.000, which is somewhat higher than that of the human C 1-inactivator (Loos et al., 1971). Two more protease inhibitors, having similar molecular weights have been found in guinea pig serum (Kobayashi & Nagasawa, 1974). The inhibitor having albumin mobility in electrophoresis appeared to correspond to the α_1 -protease inhibitor found in other mammals, since it showed both trypsin and chymotrypsin inhibition, whereas the slower-moving inhibitor only showed trypsin inhibition. In addition, a

a guinea pig antithrombin III having properties common to the other mammalian antithrombins has been isolated (Heck et al., 1979).

The α_1 -antitrypsin that was isolated from the serum of the rhesus monkey was found to be microheterogeneous and, using a broad spectrum of biochemical methods, appeared to be similar to human α_1 -antitrypsin (Berninger & Mathis, 1976).

α_1 -Antitrypsin has also been isolated from the sheep, according to a method developed for isolating human α_1 -antitrypsin (Dziegielewska & Saunders, 1981). In fact, there appears to be two α_1 -protease inhibitors having somewhat different inhibition characteristics with regards to trypsin and chymotrypsin (Juneja & Gahne, 1980a) in the sheep.

2.1.8 Conclusion

Protease inhibitors are present in the blood of the vertebrates. The gross electrophoretic pattern of these inhibitors appears to be similar in all of the species studied, with trypsin and chymotrypsin inhibitors predominantly found in the α_1 - and α_2 -regions, and occasionally also in the β -region, upon gel electrophoresis (Fossum, 1970; Nakamura et al., 1972). However, the individual plasma/serum protease inhibitors have been thoroughly studied only in the mammals. From these investigations, as reviewed above, it appears as if an α -macroglobulin inhibitor, an α_1 -protease inhibitor (α_1 -antitrypsin), and an antithrombin III are present in all mammals that have been investigated. In the different species, each one of these inhibitors, according to protease inhibition spectra, physicochemical properties, and degree of genetic polymorphism (α_1 -protease inhibitor) show similar properties.

Two antigenically distinct α -macroglobulins, but having similar physicochemical and inhibition properties, are found in many species, e.g., the pig, dog, rat and rabbit. Only one α -macroglobulin is found in man, but it does show some microheterogeneity. The reasons for the presence of two α -macroglobulins with similar properties in some species seems obscure, but differences in their ontogenetic development and their presence during various physicopathological conditions might indicate

differences in their functions in these species. Significant immunological cross-reactivity exists between the mammalian serum α -macroglobulins, while no such reactivity has been found using sera obtained from the lower vertebrates (James, 1965; Shortridge et al., 1976; Weström et al. in prep.). This indicates that there is a pronounced structural homology between the mammalian α -macroglobulins.

Inter- α -trypsin inhibitor homologues have been found in man and the rat, and probably also in the horse and the bovine; C 1-inactivator homologues are present in man and the guinea pig, and α_2 -antiplasmin homologues in the rat and presumably also in the pig, indicating that these inhibitors are also common to the various mammalian species.

It appears as if in most species there is, in addition to α_1 -protease inhibitor and antithrombin III, at least one more protease inhibitor of similar molecular weight present in the blood serum, e.g., porcine and equine α_2 -antitrypsin, canine Pi-2, rat α_2 -inhibitor and guinea pig α_1 -antitrypsin. This inhibitor appears to have a slower electrophoretic mobility than α_1 -protease inhibitor, having in most cases α_2 -mobility; and it shows appreciable trypsin inhibition but poor chymotrypsin inhibition. The observation that serum from sheep, goat, cattle, horse and dog all showed immunological cross-reactivity with antiserum to pig α_2 -antitrypsin (Weström & Juneja unpubl.), serves to strengthen the evidence for a common α_2 -antitrypsin.

The possible human counterparts to this α_2 -antitrypsin are α_1 -antichymotrypsin and α_2 -antiplasmin. However, human α_1 -antichymotrypsin only inhibits chymotrypsin and human α_2 -antiplasmin, is present only in low amounts, contrary to what seems to be true for the α_2 -antitrypsins. It is possible that during evolution, homologous inhibitors have adapted to different requirements of different species, and thus show different inhibition spectra.

2.2 Inhibitors in mammary secretions (see also Table 2)

2.2.1 In the pig

By a chance observation, Laskowski & Laskowski (1951) detected trypsin inhibiting activity in bovine milk. Some years later, Laskowski et al. (1957) extended their studies to porcine colostrum and isolated the principal protease inhibitor in sow colostrum.

Sow colostrum protease (trypsin) inhibitor (SCTI), is a low-molecular weight inhibitor of about 18.000, as determined by gel filtration (Carlsson et al., 1974), but the true molecular weight is probably lower because it also has a relatively high carbohydrate content. SCTI is highly resistant to acidic conditions, a property which is used for isolation of the protein, and to pepsin digestion (Laskowski et al., 1957). SCTI is a potent inhibitor of trypsin, chymotrypsin and leucocyte proteinases, but does not inhibit the enzymatic activity of elastase, carboxypeptidase or pepsin (Kress et al., 1971; Stancikova et al., 1980; Weström et al. unpubl.). On agarose gel electrophoresis, SCTI shows a pronounced heterogeneity, having at least seven fractions in the γ -region (Weström et al., 1979). Kress et al. (1971) isolated four electrophoretically homogeneous fractions, and found small differences in both their amino acid content and the carbohydrate moiety. However, all fractions showed the same specific activity for trypsin. At parturition, SCTI is present in the colostrum at a concentration of about 0.5 mg/ml. However, the levels rapidly decrease and after 5 days of lactation, virtually no SCTI can be detected in the milk (V, Jensen, 1977).

It has been shown that SCTI is not the only protease inhibitor in porcine colostrum. These additional inhibitors have properties in common with the porcine serum protease inhibitors (Carlsson & Karlsson, 1972/73; Jensen, 1977; Weström et al., 1979). Recent investigations have shown that a significant amount, comprising about 10-20% of the total protease (i.e., trypsin) inhibiting activity of colostrum, emanates from the serum-type inhibitors, whereas in mature milk, virtually all the protease inhibitors are of the serum-type (See also 3.3) (V, Weström et al. unpubl.). The serum-type inhibitors appear in colostrum in amounts correlated with their respective molecular weights (V). Thus, only low amounts of the largest of the serum-type protease inhibitors, the α_2 -macroglobulins, mol. wt. 960.000, are

found in milk, comprising only 3-6% of their serum levels, whereas inter- α -trypsin inhibitor, mol. wt. 160.000, comprised 14% of its serum level; α_2 -antitrypsin, mol. wt. 58.000, comprised 27% of its serum level; and α_1 -protease inhibitor, mol. wt. 51.000, was present in porcine milk in amounts equal to 42% of its serum level. The serum-type protease inhibitors found in colostrum have apparently the same properties, as shown by electrophoretic mobility, behaviour on gel filtration, and inhibitor activity, as the corresponding inhibitors in serum, in addition to immunological identity. Probably the serum-type inhibitors are transferred from the serum to the mammary secretions of the sow (V).

2.2.2 In the cow

The principal bovine colostrum protease (trypsin) inhibitor (BCTI), has also been isolated and characterized, and was found to consist of a single polypeptide chain with a relatively large carbohydrate moiety, comprising about 40% of the inhibitor (Cechova, 1976). The molecular weight has been evaluated to be about 11.000, although a higher value, 20.000, has been obtained from gel filtration experiments, probably due to the high carbohydrate content. The inhibitor contains 67 amino acids, and the primary structure was found to contain 40% homology to the bovine basic pancreatic inhibitor (Kunitz inhibitor, Trasylol[®]). BCTI inhibits trypsin, chymotrypsin, plasmin, acrosin, and leucocyte proteinases, but does not inhibit kallikrein and thrombin (Cechova, 1976; Stancikova et al., 1980).

Similar to porcine CTI, bovine CTI is heterogeneous on agarose gel electrophoresis, showing several fractions in the α -region (Pineiro et al., 1975; von Fellenberg & Horber, 1980). Seven fractions of BCTI have been isolated by ion-exchange chromatography on DEAE-cellulose, and differences in the primary structure and the carbohydrate moiety have been detected between these fractions (Tschesche et al., 1975; Jonakova & Cechova, 1977). Anti-serum to bovine CTI cross-reacts with porcine CTI (Veselsky et al., 1978). Using immunofluorescence, BCTI could only be demonstrated in the secretory epithelium of the mammary gland secreting colostrum (Veselsky et al., 1978). The total trypsin inhibiting capacity, and also the concentration of BCTI, rapidly decreases in colostrum to about one-twentieth of its original level within two days after parturition (von Fellenberg & Horber, 1980).

There are indications that two more protease inhibitors are present in bovine colostrum. These have different molecular weights and electrophoretic mobilities, and are presumably serum-type inhibitors in bovine colostrum (Barkholt-Pedersen et al., 1971; von Fellenberg & Horber, 1980).

2.2.3 In man

A comparatively low trypsin inhibiting activity has been found in human colostrum, equivalent to one-tenth or one-seventieth of the activity found in bovine or porcine colostrum, respectively (Laskowski & Laskowski, 1951). No low-molecular weight protease inhibitors can be demonstrated in human colostrum, and the inhibitory activity that was present was found to be due to what appeared to be serum-type inhibitors (Barkholt-Pedersen et al., 1971). Recently, the main protease inhibitors in human mammary secretions were found to be α_1 -antichymotrypsin and α_1 -antitrypsin (Lindberg, 1979; Lindberg et al., 1981). Especially high levels of α_1 -antichymotrypsin were observed, reaching about 130% of the normal serum level, which indicated an accumulation of this inhibitor in colostrum. The major fraction of α_1 -antichymotrypsin had a slower electrophoretic mobility migrating in the β -region, as compared to the slow α_1 -mobility for the inhibitor in the serum. No, or only trace amounts of other serum protease inhibitors or antileukoproteases could be demonstrated in human colostrum or milk (Lindberg et al., 1981).

Surprisingly, although protease inhibitors could be detected using immunological methods, no protease inhibiting activity could be demonstrated in about one-third of the mammary secretions collected from the first to the third day of lactation (Lindberg et al., 1981). Therefore, inactive inhibitors are present in these samples. The existence of proteolytic activity in the samples may indicate that the inhibitors have been consumed by proteases in the secretions. Milk obtained from all the later stages of lactation showed low but significant protease inhibiting activity.

2.2.4 In the rat

The trypsin inhibitors found in rat milk were studied by electrophoresis, after undergoing gel filtration on Sephadex G-200 (Weström & Carlsson, 1976). Main trypsin inhibiting activity was found in the third gel filtration peak, representing α_1 -antitrypsin and the two as yet unidentified protease inhibitors having α_2 - and β -electrophoretic mobility. In addition, small amounts of what appears to be α_1 -inhibitor III and α_1 -macroglobulin could be detected in the rat milk.

2.2.5 Conclusion

It appears as if there is a correlation between the quantity and quality of the protease inhibitors found in the mammary secretions, and the manner of antibody transmission from the mother to the fetus or the neonate. Thus, a relatively low inhibitor content of serum-type inhibitors are found in the colostrum/milk of species having transplacental transmission of immune protection from mother to fetus (e.g., man), or postnatal intestinal transmission of antibodies from milk via a selective route (e.g., the rat). The presence of a high inhibitor content, primarily in the form of a specific colostrum protease inhibitor, is confined to those species where antibodies are more or less non-selectively transferred to the neonate via colostrum ingestion and subsequent intestinal absorption (e.g., the cow and the pig). Serum-type inhibitors are also present in the colostrum and milk of these latter species, and the presence of the serum-type inhibitors, preferentially those of lower molecular weight, seem to be universal in mammalian milk.

3. DEVELOPMENTAL CHANGES OF THE PROTEASE INHIBITORS

3.1 In blood serum

3.1.1 In the pig

High trypsin inhibiting activities are found in the blood serum of fetal and newborn pigs, both in comparison to the adult pig and to other fetal mammals, such as man and the rat (Carlsson & Karlsson, 1972). These high levels are not due to overall increased levels of the individual serum protease inhibitors, but is instead due to the remarkably high levels of the serum inhibitors having a relatively low molecular weight (IV). Thus, at 40-50 days of fetal development, which was the earliest fetal stages possible to study, the levels of α_1 -protease inhibitor is more than 15 times that of the adult, and the levels of α_2 -antitrypsin about 4 times greater. The level of α_1 -protease inhibitor steadily decreases during the fetal period, to about 300% of the adult level at birth, whereas α_2 -antitrypsin moderately increases to 500% of the adult level at birth. The decrease in α_1 -protease inhibitor may be interpreted as a decreasing synthesis by the degenerating yolk sack during the fetal period. After birth, the levels of both α_1 -protease inhibitor and α_2 -antitrypsin decrease, and attain the adult levels after 12 and 25 days, respectively. This decrease is especially marked during the first day of life, and can partly be explained by the dilution of the plasma proteins as a result of the increase in plasma volume by 30% during the first day after the pig begins to suckle (McCance & Widdowson, 1959; Ramirez et al., 1963). However, since these inhibitors decrease by more than 30%, this may indicate that they are also being consumed by proteases.

The levels of the high molecular weight serum inhibitors, α_2 -macroglobulin f and s, are very low during the fetal period, but begin to increase near term, reaching adult levels by about 25 days after birth (IV). The increase in α_2 -macroglobulin s is especially marked during the first days after birth and, though all the other inhibitors decrease in concentration during the first day, α_2 -macroglobulin s increases. This must represent a synthesis starting near term, and probably located in the liver, since the liver, at least in man and the rat, has the capacity to synthesize α -macroglobulins (Gitlin & Gitlin, 1975; Kirkcaldy & Beaton, 1977). It has been suggested

that α_2 -macroglobulin s might serve as a marker of maturity and viability of the neonatal piglet (Weström et al., 1980).

Inter- α -trypsin inhibitor, the porcine serum protease inhibitor having an intermediate molecular weight, attains about half of the adult serum level during the fetal period (IV). After an initial decrease on the first day of life, the level of inter- α -trypsin inhibitor increases again, and the adult level is reached after about 20 days.

During the first days after birth, small amounts of SCTI appear in the serum of the suckling piglets (III). This SCTI has been absorbed from ingested colostrum but is rapidly eliminated from the bloodstream due to its low molecular weight and can be found in the urine of the piglet (Carlsson et al., 1974).

3.1.2 In other mammals

Relatively little investigation on the development of the protease inhibitors during ontogeny in species other than the pig have been undertaken. In man, the level of α_1 -antitrypsin (α_1 -protease inhibitor) is about 70% of the normal adult level at 10 weeks gestation, whereafter it increases slowly to about 150% of the adult level at term (Gitlin & Gitlin, 1975). During the postnatal period, the α_1 -antitrypsin concentration decreases, and the adult values are reached within two weeks. The liver is thought to be the principal source of α_1 -antitrypsin, but synthesis in the yolk sack during early fetal development also occurs.

High levels of α_1 -antitrypsin, which subsequently decrease throughout the fetal period similar to the pattern of development in the pig, have also been demonstrated in the fetal lamb (Dziegielwska et al., 1980).

In the human conceptus, at 10 weeks gestation, the level of α_2 -macroglobulin is about 15% of the normal adult level (Gitlin & Gitlin, 1975). It then rises exponentially to about 150% of the adult level by 30 weeks gestation. This level is maintained until term and throughout the postnatal period. The liver and the blood cells are suggested as the sources of human α_2 -macroglobulin.

In the rat, low levels of α_1 -macroglobulin and high levels of α_2 -acute-phase macroglobulin are present during the fetal period (Weström & Carlsson, 1976). During the postnatal period, α_1 -macroglobulin concentration progressively increases, whereas the α_2 -acute-phase macroglobulin concentration decreases and has almost disappeared by the time the rat has reached adulthood.

The diversity in the development of these inhibitors during ontogeny indicates that there is a difference in their importance and function in the different species. Thus, the high levels of α_1 -protease inhibitor found in the ungulates studied, i.e., the pig and the sheep, suggests that this protease inhibitor fulfills an important function in these animals during the fetal and neonatal period.

In man, rat, and mouse, relatively low levels of antithrombin III, comprising about 30-40% of the adult levels, are found during the fetal period (Hathaway et al., 1978; Lamontagne & Gauldie, 1980). After birth during the postnatal period, the concentration of antithrombin III increases until the adult level is reached. This similarity in development between man, rat and mouse, suggests that antithrombin III plays a similar role in the control of coagulation among these species during the pre- and postnatal periods.

3.2 In amniotic fluid

The porcine fetus is virtually bathed in protease inhibitors, since significant amounts of all of the inhibitors, except α_2 -macroglobulin, are found in porcine amniotic fluid (IV). These inhibitors all attain maximum levels at about mid-gestation, whereafter they decrease until term. At mid-gestation, especially high levels, about 200% of the adult serum level, of α_1 -protease inhibitor are found, while α_2 -antitrypsin is present in a concentration corresponding to 50% of the adult serum level, inter- α -trypsin at 3% of the adult serum level, and α_2 -macroglobulin at 1.5% of the adult serum level. Thus, the development of the protease inhibitors in the amniotic fluid is independent of their development in fetal serum, although there are some indications that the inhibitors have a fetal rather than a maternal origin. In ungulates, the placenta and the amnion

TABLE 1. Summary and comparison of properties of the protease inhibitors in blood serum/plasma from different mammals.

Inhibitor	Physico-chemical properties				Inhibition activity						Immuno-cross reactivity	
	Molecular weight	Electroph. mobility	Isoel. point pH	Carbo-hydrate %	Trypsin	Chymotryp.	Elastase	Thrombin	Plasmin	Other		
HUMAN:												
α_2 -Macroglobulin	725.000/ ~ 19S	α_2	~ 5.4	8	+	+	+	+	+	+	see the other species	Laurell & Jeppsson, 1975 Heimburger, 1975 Harpell & Rosenberg, 1976 Starkey & Barrett, 1977 (reviews)
Inter- α -trypsin inhibitor	160.000/ 6.4S	inter- α		9	+	(+)	-	-	+			
CI-inactivator	105.000	α_2		35	(+)(+)	-	-	+	+			
α_1 -Protease inhibitor	50-55.000/ 3.4S	α_1	~ 4.8	12	+	+	+	-	+	+		
Antithrombin III	65.000	α_2		13	+	-	-	+	(+)	+		
α_1 -Antichymo- trypsin	69.000	α_1 (slow)		25	-	+	-	-	-			
α_2 -Antiplasmin	65-70.000/ 3.4S	α_2		12-14	(+)	-	-	+				Collen & Wiman, 1978
PORCINE:												
α_2 -Macro- globulin f	960.000- 740.000/ 19-20S	α_2 (fast)	5.4		+	+	+	+	+	+	Human α_2^M Human PZP Canine αM Rat αM	I, II Jacquot-Armand & Guinand, 1967 "-" , 1976 Tsuru et al., 1975 Martinsson & Carlström, 1977 Weström et al, in prep.
α_2 -Macro- globulin s	"-	α_2 (slow)	"-		+	+	+	+	+	+		
Inter- α -trypsin inhibitor	160.000	inter- α			+	+					Human IaI	I, II
α_1 -Protease inhibitor	51.000	$\alpha 1b-\alpha_1$			+	+	+				Human α_1 PI	I, II Baumstark, 1973 Juneja & Gahne, 1981
α_2 -Antitrypsin	58.000	α_2			+	?	-					II
β -Elastase inhibitor	50.000- 60.000	β			-	?	+					II Baumstark, 1973
Antithrombin III	58.000			16				+			Porcine ATIII Rat ATIII Rabbit ATIII	Koide, 1979
α_2 -Antiplasmin	48.000	α_2									Human AT III Human α_2 AP	I, II I, II
EQUINE:												
α_2 -Macroglobulin	~ 19S	α_2			+							Matthews, 1979
"-	19.6S	α_2			+							Kueppers, 1980
α_1 -Protease inhibitor	~60.000	α_1			+							Pellegrini & von Fellen- berg, 1980
Pr	~60.000	$\alpha 1b$			+	+				+		Ek, 1977
"-	~ 4S	"-			+							Matthews, 1979
α_1 -Antitrypsin	3.5S	α_1			+							Kueppers, 1980
Pi-1					+	+						Juneja et al., 1979
Antithrombin III	53.000	α_2		16					+		Human ATIII Bovine ATIII	Kurachi et al., 1976
α_2 -Antitrypsin	4.6S	α_2			+							Kueppers, 1980
α_2 -Protease inhibitor	~65.000	α_2			+							Pellegrini & von Fellen- berg, 1980
Xc	~ 4.5				+							Matthews, 1979
Pi-2					+	-						Juneja et al., 1979
CATTLE:												
α_2 -Macroglobulin	800.000/ 17.8S	α_2	5.1	9	+		+	+				Nagasawa et al., 1970 a,b Sugihara et al., 1971
α_1 -Antitrypsin	~45.000/ 3.5S	α_1	4.0	+	+		+	+	+	+		Tan & Gans, 1972
Acid-labile tryp- sin inhibitor	(71.000)	α_1	~ 3.9		+	?	+	+	+	+		Habermann, 1968 Wu & Laskowski, 1960

Inhibitor	Physico-chemical properties				Inhibition activity				
	Molecular weight	Electroph. mobility	Isoel. point pH	Carbo-hydrate %	Trypsin	Chymotryp. Elastase	Thrombin	Plasmin	Other
Immuo-cross reactivity									
<u>CATTLE continued:</u>									
Antithrombin III	57.000	α_2		12				+	Human ATIII Horse ATIII
"-	56.000		4.5-5.0	10			+		- Human ATIII
Protease inhibitor	72.000/ 3.55	β			+		+		
Pi-1					+	+			
Pi-2					+				
Pi-3					+	+			
<u>CANINE:</u>									
α_1 -Macroglobulin	19.45	α_2 (fast)			+	+		+	Human α_2M
α_2 -Macroglobulin	18.55	α_2 (slow)			+	+		+	Human α_2M
α_1 -Protease inhibitor	58.000	α_1	4.4-4.5	11	+	+			- Human α_1PI
α_1 -Antitrypsin		α_1			+	+		+	
"-	57.000	α			+	+			
Pi-1					+	+			
Antithrombin III	77.000						+		
Pi-2					+				
Pi-3					+	+			
<u>RAT:</u>									
α_1 -Macroglobulin	760.000/ 17.85	α_1	4.4		+	+			
"-	746.000/ 18.75	α_1	4.4	8	+				
α_2 -Acute phase macroglobulin	770.000/ 18.45		4.55	10	+	+			Human α_2M
α_2 -Macroglobulin	716.000/ 19.85	α_2	4.5	9	+				
α_2 -Acute phase macroglobulin	760.000		4.8	12	+		+		
α_1 -Inhibitor III	215.000/ 8.65	α_1	4.65	15	+	+	+		Human IaI
α_1 -Protease inhibitor	54.000		4.3-4.7	13					
α_1 -Antitrypsin	50.000/ 4.65	α_1	4.5-4.8	10	+				
"-		α_1			+	+	+		
Antithrombin III	63.000			15			+		- Human ATIII - Porcine ATIII - Rabbit ATIII
"-	64.000		4.9				+		
α_2 -Inhibitor (?)	~70.000 73.000		4.4		+	+	-	+	
β -Inhibitor	~ 70.000				+				
<u>RABBIT:</u>									
α_1 -Macroglobulin	16.65	α_1	4.5	15	+	+	+		
α_2 -Macroglobulin	850.000/ 20.35	α_2		9					- Human α_2M
"-							+		
α_1 -Antitrypsin	58.000	α_1	4.4-4.9	11-12	+				
Antithrombin III	63.000			14			+		Human ATIII Porcine ATIII Rat ATIII

Kurachi et al., 1976

Nordenman et al., 1977

Gray et al., 1960

Juneja & Gahne, 1980b

Ohlsson, 1971 a,b,c

Abrams, 1978

Ohlsson, 1971 b,c,d

Schnitzlein et al., 1980

Juneja et al., 1981

Damus & Wallace, 1974

Juneja et al., 1981

Gauthier et al., 1974

Gauthier & Mouray, 1975

Gordon, 1976

Gauthier & Mouray, 1975

"-", 1976

Gordon, 1976

Nieuwenhuizen et al., 1979

Gauthier & Ohlsson, 1978

Esnard & Gauthier, 1980

Ikehara et al., 1981

Takahare et al., 1980

Gauthier et al., 1978

Koide, 1979

Kumada & Abiko, 1980

Weström & Carlsson, 1976

Hojima et al., 1977

Weström & Carlsson, 1976

Got et al., 1965

Berthillier et al., 1968

Lebreton de Vonne, 1978

Picard et al., 1964

Lebreton de Vonne, 1978

Koj et al., 1978

Koide, 1979

TABLE 1 cont.

TABLE 1 cont.		Physico-chemical properties			Inhibition activity					Immuno-cross reactivity	
Inhibitor	Molecular weight	Electroph. mobility	Isoel. point pH	Carbo-hydrate %	Trypsin	Chymotryp.	Elastase	Thrombin	Plasmin		Other
<u>MOUSE:</u>											
α_2 -Macroglobulin	18.8S	$\alpha_2?$	5.0		+						- Human α_2M Greene et al., 1971
α_1 -Antitrypsin	58,000/ 3.5S	α_1	4.6,4.7		+						Myerowitz et al.,1972
"	53,000	α_1			+						Gauldie et al., 1980
<u>GUINEA PIG:</u>											
C1-inactivator	170,000/ 6.1S	α							+		Loos et al., 1971
α_1 -Protease inhibitor	78,000	$\alpha 1b$			+	+					Kobayashi & Nagasawa, 1974
α_1 -Antitrypsin	73,000	α_1			+	-		+	?		
Antithrombin III	64,000	α_2	5.2					+			Heck et al., 1979
<u>HAMSTER:</u>											
α_2 -Macroglobulin	725,000	α_2									Stein-Streilein & Hart, 1981
<u>RHESUS-MONKEY:</u>											
α_1 -Antitrypsin	60,000	α_1		12	+						Berninger & Mathis, 1976

Table 2. Summary and comparison of properties of protease inhibitors in mammary secretions from different mammals.

<u>PORCINE:</u>											
Colostrum protease inhibitor (SCTI)	~18.000	γ (~7 frac.)		+	+	+	-	+		Bovine CTI	Kress et al., 1971 Carlsson et al., 1974 Veselsky et al., 1978 Weström et al., 1979
α_1 -Protease inhibitor	~51.000	α_1			+	+	+			Serum α_1PI	
α_2 -Antitrypsin	~58.000	α_2				+				Serum α_2AT	V
α_2 -Macroglobulin f	~960.000	α_2 (fast)				+	+	+		Serum α_2Mf	Weström et al., unpubl.
α_2 -Macroglobulin s	~960.000	α_2 (slow)				+	+	+		Serum α_2Ms	
<u>BOVINE:</u>											
Colostrum trypsin inhibitor (CTI)	11.000	α (~4 frac.)	4.2	40	+	+	-	+	+	Porcine SCTI	Cechova, 1976 Veselsky et al., 1978 Pellegrini & von Fellenberg, 1980
α_1 -Protease inhibitor		α_1				+					Pellegrini & von Fellenberg, 1980
Serum-type inhibitor ?											
<u>HUMAN:</u>											
α_1 -Antichymotrypsin	$\alpha_1 + \beta$				-	+	-			Serum α_1AChy	Lindberg, 1979
α_1 -Antitrypsin		α_1			+	+	+			Serum α_1AT	Lindberg et al., 1981
<u>RAT:</u>											
α_1 -Antitrypsin		α_1			+						
α_2 -Inhibitor		α_2			+						
β -Inhibitor		β			+						Weström & Carlsson, 1976
α_1 -Inhibitor III		$\alpha 1b$			+						
α_1 -Macroglobulin		α_1			+						

appear to be impermeable to maternal proteins (Brambell, 1970; Morris & Barron, 1980). Furthermore, the same genetic variants of α_1 -protease inhibitor are present in paired amnionic fluid-fetal serum samples. Recent preliminary investigations have also shown that the entire gastrointestinal tract of the fetus is flooded with protease inhibitors (Ohlsson & Weström, unpubl.), and circulation of inhibitors between the amnionic fluid and the fetal gastrointestinal tract is probable. Another source of protease inhibitors in the amnionic fluid may be fetal urine.

Protease inhibitors have also been found in human amnionic fluid, but it was considered that the majority were of maternal origin (Sutcliffe, 1975; Burnett & Bradwell, 1980). The development of α_1 -protease inhibitor appears to follow that of the total proteins, with increasing levels of the inhibitor up to about 25 weeks gestation, followed by a decrease until reaching term (Evans et al., 1975; Guibaud et al., 1975). Only low levels of α_1 -protease inhibitor, as compared to those of the pig, and comprising only about 10% of the adult human serum level, have been observed. Interestingly, Burnett & Bradwell (1980), found what appeared to be complexes of α_1 -anti-chymotrypsin and proteases in human amnionic fluid.

3.3 In mammary secretions

In porcine colostrum, the high trypsin inhibiting capacity (TIC), and the level of SCTI, rapidly decrease in parallel during the first day of lactation to about 30% of their initial values in presuckled colostrum (V, Laskowski et al., 1957; Jensen & Pedersen, 1979). During the following days, they continue to decrease; at five days lactation SCTI cannot be detected in milk, and only about 1% of the original TIC remains. During the change from colostrum to milk formation, the synthesis of SCTI ceases; therefore, SCTI can be considered as being a true colostrum protein. The factors regulating the synthesis of SCTI are completely unknown. However, SCTI is already synthesized during late pregnancy, since it can be detected in the urine of the pregnant sow (Weström & Svendsen, unpubl.).

The levels of the serum-type protease inhibitors in porcine mammary secretions also decrease during the first days of lactation. After five days, only 1-4% of the adult serum levels of the different inhibitors remain in

milk. The decrease in the concentration of the α -macroglobulins is slower than the decrease in albumin, which represents the overall protein decrease in mammary secretions. Therefore, the α_2 -macroglobulins constitute an increasing fraction of the decreasing concentration of protease inhibitors in the mammary secretions of the sow.

A similar dramatic decrease in the TIC during the first days of lactation has also been observed in other ungulates, such as the cow, the ewe and the mare (Sandholm & Honkanen-Buzalski, 1979), and must also represent the decrease in the specific colostrum protease inhibitor in these species, at least in the cow (Veselsky et al., 1978).

The levels of the protease inhibitors in human mammary secretions, α_1 -antichymotrypsin and α_1 -protease inhibitor, decrease markedly during the first week of lactation (Lindberg, 1979; Lindberg et al., 1981). This decrease coincides with the overall decrease in protein concentration during the colostrum/milk formation. Although relatively high concentrations of these inhibitors are present during the first three days of lactation, the inhibitors are inactive in about one-third of the milk samples, which results in a low protease inhibiting capacity in these early milk samples (Laskowski & Laskowski, 1951; Hyanek et al., 1965).

In the rat, the TIC increases in colostrum during the first days of lactation, followed by a slow decrease in the milk (Carlsson et al., 1975).

In conclusion, the levels of the serum-type protease inhibitors which are present in the mammary secretions of all of the mammalian species studied, decrease at a rate similar to that of most proteins in colostrum/milk during the lactation period. However, in some species, these inhibitors may be inactive during the first days of lactation. The specific colostrum protease inhibitor which is present in the ungulates, decreases rapidly in colostrum and does not appear to be present in mature milk.

4. PHYSIOLOGICAL FUNCTIONS OF THE PROTEASE INHIBITORS

4.1 Blood serum inhibitors

The main physiological role of the mammalian blood serum protease inhibitors is, in the broad sense, understood, i.e., the elimination of undesired proteolysis intravascularly as well as extravascularly. However, only limited information about the detailed functions of the various inhibitors is available, and these come mostly from studies in man.

A complicated system of blood proteases participate in the processes of blood coagulation, fibrinolysis, complement activation, and the kallikrein-kinin system. The activity of the enzymes in these systems are partially controlled by such serum inhibitors as antithrombin III, α_2 -antiplasmin, C 1-inactivator and the α_2 -macroglobulins (Heimbürger, 1975; Harpel & Rosenberg, 1976; Collen & Wiman, 1978).

Another function of the protease inhibitors, primarily the α -macroglobulins, α_1 -protease inhibitor and α_1 -antichymotrypsin, is to eliminate proteases released during infections and inflammation (Heimbürger, 1975; Starkey & Barrett, 1977). Inactivation of proteases of exogenous origin, e.g., from microorganisms, is a function both of α_1 -protease inhibitor and the α -macroglobulins, because of their broad specificity of enzyme inhibition. This is especially true for the α -macroglobulins, which can inhibit enzymes of all the functional classes. Moreover, endogenous proteases released from leucocytes and the pancreas during inflammation, are also inactivated by the protease inhibitors (Ohlsson & Ohlsson, 1977; Balldin & Ohlsson, 1979).

The α -macroglobulins appear to play a central role in the elimination of active proteases. Upon binding with the protease, a conformational change takes place in the α -macroglobulin molecule which results in the protease becoming entrapped within the complex (Starkey & Barrett, 1977). These complexes are then rapidly removed from the circulation by reticuloendothelial cells (Ohlsson, 1971e). In addition, a transfer of proteases from α_1 -antitrypsin to the α -macroglobulins has been shown to occur (Ohlsson & Laurell, 1976; Balldin et al., 1978), indicating that α_1 -protease inhibitor has a carrier function, and the α -macroglobulins acts as a central eliminator. In the pig, the α -macroglobulins probably have a similar function, since the porcine α -macroglobulins have the highest relative affinity for

trypsin, chymotrypsin and elastase, of all the inhibitors in porcine serum tested in vitro (Ohlsson et al., 1981).

Since the α -macroglobulins are too large to escape from the circulation in appreciable amounts during normal conditions, the eliminating function of these protease inhibitors has mostly been considered to be intravascular. However, during inflammation, increased vascular permeability allows the α -macroglobulins to leave the circulation. In addition, a local production of α -macroglobulins has been observed in connective tissue (Cassiman et al., 1980), thus these inhibitors may also have some local functions in the tissues of the body.

Proteolysis under normal physiological conditions is a fundamental biochemical reaction in protein turnover and protein "processing", and there may be an extracellular release of enzymes during these processes. Extensive enzymatic degradation of proteins also occur as an integral part of food digestion in the intestines, and proteolytic enzymes may escape from the intestines. Using radioimmunoassay techniques, it has recently been shown that proteases of pancreatic origin can be detected in the blood circulation (Borgström, 1979; Geokas et al., 1979a, b). These proteases exist either in their zymogen form or in complexes with the appropriate serum protease inhibitor. The pancreatic proteases may reach the bloodstream via direct secretion from the pancreas (Borgström, 1979; Geokas et al., 1979a), or via absorption from the intestines (Ambrus et al., 1967; Genell et al., 1977; Lake-Bakaar et al., 1980). Recirculation of pancreatic proteases has also been suggested (Liebow & Rothman, 1975; Lake-Bakaar, 1980), but is a controversial subject (Rohr et al., 1981). If active protease is absorbed via the intestine, which appears reasonable to assume since significant amounts of food protein can be absorbed intact, or is absorbed as large breakdown products by the adult gut (Warshaw et al., 1974; Hemmings 1981), these proteases could be locally inactivated by the serum protease inhibitors in the gut or in the circulation.

The high levels of serum protease inhibitors observed in the fetal and neonatal piglet suggest that there is a special need for protection of the organism from proteolytic activity during this period of development in the pig. The need for protection against the potential absorption of proteases from the gut appears probable (VI), since ungulates are known to absorb

undegraded proteins non-selectively from the gut to the blood serum during this period, and especially during the first days after birth when receiving colostrum (III, Brambell, 1970). In the piglet, pancreatic proteases are present and active at birth, and a capacity for intestinal proteolysis at this time has also been shown (Hardy, 1969; see also 4.2.2).

Little is known about the presence of pancreatic proteases in the gut during the fetal period, but investigations are in progress at our laboratory. Preliminary studies have shown that the entire gastrointestinal tract in the fetal piglet is flooded with serum protease inhibitors (Ohlsson & Weström, unpubl.). Since the amniotic fluid also contains large amounts of protease inhibitors, circulation between the gastrointestinal tract and the amniotic fluid appears probable, constituting a significant and dynamic capacity for inhibiting the action of proteases.

The relatively low levels of serum protease inhibitors found during the fetal and neonatal period in man and in the rat, in comparison to the pig and to the sheep (see also 4.1.2), might be related to the lower transmission of protein via the intestine in these mammals (Brambell, 1970). Therefore, the need for protection against proteases which may be absorbed from the gut in these animals should also be much less.

Neutralizing proteases from the gastrointestinal tract during development in the ungulates might not be the only function of the serum protease inhibitors. It has been suggested that the high levels of sheep α_1 -antitrypsin are related to the rapid turnover of cells which occurs in developing tissues, and thus the presence of the protease inhibitor serves as a potential protection against release of proteases from the cells (Dziegielewska & Saunders, 1981).

4.2. Inhibitors in mammary secretions

The physiological functions of the protease inhibitors in the mammary secretions may be exerted both in the mammary gland of the lactating female and in her nursing offspring.

4.2.1. Function in the mammary gland

The general function of the protease inhibitors in the mammary gland is believed to be protection of the mammary tissue and milk proteins from possible proteolytic damage (V, Stancikova et al., 1980; von Fellenberg & Hober, 1980; Honkanen-Buzalski & Sandholm, 1981; Lindberg et al., 1981). Since this should be a function common to all mammals, this protection must be exerted primarily by the serum-type protease inhibitors because these apparently are present in the mammary secretions of all mammals. Protection of the mammary tissues has also been ascribed to the specific colostrum protease inhibitor (CTI) found in the ungulates (Stancikova et al., 1980; von Fellenberg & Hober, 1980), but it seems more probable that the principal function of CTI is coupled to enhancing the intestinal absorption of undegraded proteins, which is a common mechanism among newborn ungulates (see also 4.2.2).

Since the highest levels of the protease inhibitors are found in colostrum, it seems probable that they exert an important function during colostrum formation, when there is a pronounced turnover of epithelial cells. Evidence in favour of this function is the detection of inactive protease inhibitors and protease-antiprotease complexes in about one-third of human colostrum samples (Lindberg et al., 1981). In addition, protease activity of unknown origin can be observed in these samples. Preliminary observations have revealed the presence of immunoreactive anodal trypsin (Lindberg et al. unpubl.). Increased amounts of the serum-type protease inhibitors have also been observed in milk from cows with mastitis (Honkanen-Buzalski & Sandholm, 1981) and milk from humans with mastitis (Lindberg et al. prel. results). These inhibitors have the ability to protect the mammary gland from proteolytic enzymes liberated from, e.g., polymorphonuclear leucocytes, which are known to invade the milk alveoli during intramammary infections.

4.2.2. Function in the nursing offspring

The placenta of the ungulates, e.g. the cow and pig, does not allow passage of serum proteins from mother to fetus, and the newborn of these species are born devoid of protecting immunoglobulins (Brambell, 1970).

Therefore, the newborns must rely on a postnatal intestinal transfer of immunoglobulins and possibly other proteins, from the colostrum to ensure their survival. In the original study of Laskowski & Laskowski (1951) on colostrum trypsin inhibitors, these workers coupled the observation that proteins could escape proteolytic digestion in the newborn during protein transmission in the intestinal tract, with the presence of protease inhibitors in the colostrum. Several attempts have been made to establish this function of colostrum protease inhibitors, mainly using the piglet (see review by Jensen, 1978), but these studies were not conclusive.

Recently, the role of protease inhibitors in the colostrum could be more precisely defined by studying the effect of the natural content of these inhibitors in sow colostrum on the intestinal absorption of immunoglobulins, as well as on the absorption of other colostral proteins (III, Jensen & Pedersen, 1979; Weström et al., 1979). Both trypsin and chymotrypsin have been shown to be present in the pancreas of the newborn piglet (Gorrill & Friend, 1970; Pond et al., 1971; Ohlsson & Weström, unpubl.). The newborn pig is capable of extensively proteolyzing bovine γ -globulin when fed in the absence of a protease inhibitor (Hardy, 1969). Therefore, the presence of porcine colostrum protease inhibitors result in the increased efficiency of transmission of undegraded proteins, apparently by reducing the proteolytic activity in the gastrointestinal tract of the newborn pig.

The specific colostrum protease inhibitor (CTI) present in the colostrum of the ungulates must primarily be responsible for the effect on intestinal proteolysis, since it is present as the dominating inhibitor in colostrum; but the serum-type inhibitors may have some complementary function, perhaps due to their broader enzyme specificity. The ungulates appear to have evolved their specific colostrum inhibitors from the basic pancreatic trypsin inhibitor, or from a common ancestor, since they show similar properties and sequence homology, in order to meet the need for an effective post-natal transmission of proteins via the intestinal tract from mother to young.

Since it has been observed that significant amounts of undegraded colostrum proteins still can be transmitted via the intestines even when no inhibiting activity was present in colostrum (III), other factors must also be involved in the regulation of the intestinal protein absorption. One contributing factor may be the huge amounts of various proteins which the neonate

acquires when ingesting colostrum. Hardy (1969) observed an increased absorption of protein when the concentration of the protein fed was increased. Another factor might be the presence of serum protease inhibitors in the gastrointestinal tract at birth (Ohlsson & Weström, unpubl.), which probably exert a considerable inhibiting capacity for proteases that may be released from the pancreas.

5. SUMMARY

Protease inhibitors have been identified and biochemically characterized in porcine blood serum, amniotic fluid, and mammary secretions, with emphasis on their development during the ontogeny of the pig and during the lactation period of the sow. These results have made it possible to determine some of the physiological functions of the protease inhibitors in the growing pig.

At least seven protease inhibitors have been identified in adult porcine serum, according to differences in molecular weight, as estimated by Sephadex G-200 gel filtration; electrophoretic mobility on agarose gel electrophoresis; specificity of protease inhibition; and immuno-cross-reactions with human protease inhibitors. Similarities exist between most of the porcine and human serum inhibitors and those of other mammalian species, i.e., α -macroglobulins, inter- α -trypsin inhibitor, α_1 -protease inhibitor, antithrombin III, and α_2 -antiplasmin. However, in comparison to man, some differences are also evident, i.e., the existence of two porcine α -macroglobulins having slightly different electrophoretic mobilities (α_2 -macroglobulin f and s), and an apparently specific porcine trypsin inhibitor, α_2 -antitrypsin. However, these inhibitors do appear to have counterparts in other animals.

High levels of the serum inhibitors, α_1 -protease inhibitor and α_2 -antitrypsin, are present during the fetal and neonatal life of the piglet, whereas the levels of the α_2 -macroglobulins are very low. Near term, the levels of the α_2 -macroglobulins start to increase. This is especially pronounced in the case of α_2 -macroglobulin s, indicating that there is an extensive synthesis of the α_2 -macroglobulins. After birth, the levels of α_1 -protease inhibitor and α_2 -antitrypsin markedly decrease, and the adult levels of all serum inhibitors are reached within 12-35 days.

The high total serum protease inhibiting capacity ~~in colostrum~~ and its sudden decrease immediately after the pig begins to receive colostrum after birth, is primarily due to the changes in the levels of α_1 -protease inhibitor and α_2 -antitrypsin. This suggests that these inhibitors have an important function during fetal development and the first days of neonatal life. Since the piglet nonselectively absorbs undegraded proteins from the gastrointestinal tract during this period, in common with other ungulates,

protection from active intestinal proteases would be a probable function of these protease inhibitors.

In porcine amniotic fluid, the highest concentrations of the protease inhibitors are reached at mid-gestation. The level of α_1 -protease inhibitor is especially high, equivalent to 200% of the adult serum level, whereas α_2 -antitrypsin comprises 50%, inter- α -trypsin inhibitor comprises 3%, and α_2 -macroglobulin f comprises 1.5% of the adult porcine serum levels. The porcine inhibitors present in amniotic fluid appear to be of fetal origin rather than maternal origin contrary to that proposed for the protease inhibitors present in human amniotic fluid.

The high total protease (trypsin) inhibiting capacity in porcine colostrum is mainly due to the presence of the specific colostrum protease inhibitor (SCTI), but 10-20% of the inhibiting capacity emanates from the presence of serum-type protease inhibitors. The latter types of inhibitors are present in colostrum in reverse relation to their molecular weights, ranging from 42% of the adult serum level for the inhibitor with the lowest molecular weight, α_1 -protease inhibitor, to 3-6% for the α -macroglobulins.

The protease inhibiting capacity rapidly decreases in the mammary secretions during the first days of lactation. After five days, only 1% of the original inhibiting capacity remains, and no SCTI can be detected. Although the levels of the serum-type protease inhibitors also decrease, they are responsible for the remaining low inhibition capacity that can be detected in mature milk.

The protease inhibitors in porcine milk, primarily those of the serum-type, since these appear to be common to all mammals, constitute a protection for the mammary gland from possible proteolytic attack. This protection may be of particular importance during colostrum formation, when the epithelial cell turnover is pronounced, and during mastitis, when the mammary gland is invaded by leucocytes and the probability of proteases escaping or being released from the respective cells is high.

By feeding newborn pigs colostrum in which the protease inhibiting capacity was eliminated by the addition of trypsin, it was conclusively proven that the presence of the colostrum protease inhibitors increases the quantity

and the quality of the intestinal transmission of undegraded colostrum proteins into the blood circulation in the newborn pig. This effect must mainly be due to the trypsin-chymotrypsin inhibition in the intestines of the pig which was brought about by the specific colostrum protease inhibitors (SCTI). It is tempting to suggest that the specific colostrum protease inhibitors have evolved in the ungulates because of the need for an effective transmission of undigested proteins/antibodies across the intestinal tract in the early postnatal period.

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